

Use of Fat Co- and By-products in Poultry Nutrition

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Introduction

A normal practise in broiler formulation is the addition of fat to feed in order to achieve a high nutrient concentration. Fat materials obtained from by-products and co-products of the food chain are important feedingstuffs for feed producers and help to decrease environmental charges. Nevertheless, the origin and quality of these fats are quite variable and often not well regulated or controlled.

The characterization of composition, quality and contamination of fat blends used in animal feeding is one of the most important key points in the assessment of quality and safety of meat production. Moreover, it is very important to determine the ability of these fats to modify lipid composition and the level of oxidation of meat. In parallel, assessing and quantifying the transfer rate of some undesirable products (fat degradation and contamination compounds) from these fats to the corresponding meat and other animal tissues is necessary to prevent public health risks.

The accumulative and persistent undesirable contaminants undergo a *biomagnification* effect, increasing their concentration throughout the food chain. The term 'dioxins' covers a group of 75 polychlorinated dibenzo-p-dioxin congeners (PCDD) and 135 polychlorinated dibenzofuran (PCDF) congeners, of which 17 are of toxicological concern. Polychlorinated biphenyls (PCBs) are a group of 209 different congeners which can be divided into two groups according to their toxicological properties: 12 congeners exhibit similar toxicological properties to dioxins and are therefore often termed 'dioxin-like PCBs'. Each congener of dioxins or dioxin-like PCBs exhibits a different level of toxicity. In order to be able to sum up the toxicity of these different congeners, the concept of toxic equivalent factors (TEFs) has been introduced to facilitate risk assessment and regulatory control. This means that the analytical results relating to all 17 individual dioxin congeners and to the 12 dioxin-like PCB congeners are expressed in terms of a quantifiable unit, namely the 'TCDD toxic equivalent concentration' (TEQ). The most important characteristics of dioxins and dioxin-like PCB compounds are that they are very liposoluble and poorly degradable. For these reasons, animal tissues are those materials which permit greater accumulation of such substances. The main cause of human exposure to these toxic compounds is the food chain (>95%). For this reason, the basic criterion of not introducing into the food chain more ingredients (for animal or human foodstuffs) which tend to increase these exposure levels must be adopted, including the introduction of methods to help reduce these levels. Consequently, with regard to fats used in the manufacture of feeds, the content of these contaminants must be controlled very strictly. In this respect, the European Commission decisions include specific bans against products for animal feeding such as marine oil contaminated with more than 6.0 ng WHO-PCDD/F-TEQ/Kg oil or 24.0 ng WHO-PCDD/F-PCB-TEQ/Kg oil (Directive 2006/13/EC) and against products for human consumption such as chicken meat containing more than 2.0 pg WHO-PCDD/F-TEQ/g fat or 4.0 pg WHO-PCDD/F-PCB-TEQ/g fat (Regulation (EC) N° 199/2006).

Other liposoluble compounds accumulating in foods are polycyclic aromatic hydrocarbons (PAHs). PAHs constitute a large class of organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms; the most studied is benzo[a]pyrene, which is often used as a marker for PAH in ambient air and food. PAHs are also a group of substances which may

constitute a significant public health problem, being potentially genotoxic and carcinogenic to humans, and food constitutes the main cause of exposure to them, except for the smoking population. The origin of PAHs in foods is mainly due to the method of food preparation, although their origin may also be traced to pollution of the environment and deposition in plants. The presence of PAHs in foods is estimated in ppb ($\mu\text{g kg}^{-1}$). But PAHs, being contaminants known as *inevitable* (difficult to eliminate) cannot be realistically regulated (EFSA, 2002). However, the estimated maximum daily intake of benzo[a]pyrene from food is approximately 420 ng benzo[a]pyrene per person.

Fats and oils used in animal feeding can undergo different chemical degradations during processing and storage, which are mainly oxidation, polymerization and isomerization. The products generated by these chemical degradations can decrease the fat nutritional value and give rise to toxic compounds. Some products coming from oil degradation during industrial processes, frying or cooking, can also show some toxicity. Many of these compounds are dimers, trimers and other polymers, whose presence may reach up to 30% of total oil in cooking oils. Other components of interest are cyclic monomers (that have higher toxicity compared with polymers) mainly due to their higher rates of absorption in the body. The presence in feeding fats of oxidised FAs and TG (peroxides, epoxides, etc.) are less relevant, because they are minority and unstable and break down to produce other compounds, called *secondary oxidation* compounds (Boatella et al., 2000). All these compounds have unclear harmful characteristics. Some processes, such as heating, refining and particularly hydrogenation can raise the contents of fatty acid isomers, whose negative health effects are well established. Finally, mention need be made of sterol oxide formation. In this field, the study of the oxidation of cholesterol is fairly well advanced and harmful effects of these compounds on the body have been detected, especially those effects related to atherosclerosis development. In this area, a large number of studies show the influence of feeding fat on the formation of cholesterol oxidation products in meats, on the fatty acid oxidation, and on the deposit of *trans* and conjugated isomer fatty acids.

For all the above, one of the main objectives of the present work is to study the transfer of contaminants and altered products from feeding fats to chicken meat. This work is developed within the European Project entitled "Quality and safety of feeding fats obtained from co-products or by-products from the food chain" - **Feeding Fats Safety** www.ub.es/feedfat/. The primary aim of this research project is for animal nutrition requirements to have a high level of safety and quality of some types of meat production, on the basis of the use of fats coming from by-products or co-products of the food chain. This aim must be adequate for consumer satisfaction and health demands, and must have environmental protection.

The most relevant data related to using fat co- and by-product materials recycled from the food chain as chicken feedstuffs are now presented, and special attention is paid to the transfer rate of certain undesirable products (fat degradation and contamination compounds) from these fats to the corresponding poultry meat; in particular, the dioxines, PCBs, PBDEs, PAHs, *trans* FA and oxidation compound levels. The implications on consumer health are discussed.

Working Plan

First of all, characterization and classification of feeding fats used in the European market that come from co- and by-product materials of the oil and fat industry were made (a summary of the main results on fat characterization can be found at the web site of the project). From these results, different fats and oils were selected, and 4 chicken trials were conducted to assess the rate of transfer from feeds to meat of selected contaminant and degradation compounds, which were chosen according to their higher quality and safety interest.

The experiments were performed in the Animal Experimental Unit of the Veterinary School of the Universidad Autonoma de Barcelona (UAB) and received prior approval from the respective Animal Protocol Review Committee of the UAB. All animal housing and husbandry conformed to European Union guidelines.

In each experiment, 64 female broilers Ross 308 were randomly distributed into two dietary treatments and allocated into 16 cages (8 replicates/treatment). The dietary treatment consisted of a common basal diet supplemented with 6 % of recycled fat, differing in level of alteration high (H), and low (L) (table 1). The four different compounds studied in the added fat were: *Trans* fatty acids (TH=10.01 % and TL=0.14 %); dioxin and polychlorinated biphenyls PCBs (HC=9.78 pg TEQD and 19.02 pg TEQPCB/g of oil and LC=1.95 pg TEQD and 7.69 pg TEQPCB/g of oil); polycyclic aromatic hydrocarbons PAHs (HP=5.291 µg/g of oil and LP< 2 µg/g of oil) and, oxidation products (HO= 67.43 p-anisidine and LO=2.74 p-anisidine) (table 2). The experimental feeds were manufactured in the Animal Science Department, Polytechnic University of Valencia, Spain.

Table 1. Ingredients and nutrient composition of the basal diet.

Ingredient (%)		Nutrient Composition (%)	
Corn	52.7	Metabolisable Energy (kcal/kg)	2,892
Soybean Meal (47% of CP)	30.0	Dry matter	88.3
Added Fat Material	6.0	Crude protein	20.9
Full-fat Soybean	6.0	Ether Extract	9.6
HCl L-Lysine	0.3	Crude Fibre	3.0
DL-Methionine (99%)	0.2	Ash	6.5
Dicalcium Phosphate	2.5		
Calcium Carbonate	1.3		
Salt	0.5		
Vitamin and mineral premix ¹	0.5		

¹Composition of vitamin and mineral premix (1 kg of feed contained): Vitamin A: 6000 UI; Vitamin D3: 1200 UI; Vitamin E: 10 mg; Vitamin K₃: 1.5 mg; Vitamin B₁: 1.1 mg; Vitamin B₂: 4 mg; Vitamin B₆: 1.5 mg; Vitamin B₁₂: 9 µg; Folic acid: 4 mg; Biotin: 50 µg; Panthothenic acid: 6 mg; Nicotinic acid: 21 mg; Coline: 360 mg; Mn: 75 mg; Zn: 50 mg; I: 0,18 mg; Fe: 30 mg; Cu: 6 mg; Se: 0.2 mg; Co: 0.2; Etoxiquin: 16 mg. Addition of Coline Clorure 15 mg.

At 47 days of age, animals were sacrificed in a commercial slaughter house, legs were deboned and meat coming from the 4 animals of each replicate (8 replicates/treatment) was ground in a mixer until a suitable homogeneous sample was obtained, then representative aliquots for each analysis were vacuum packed, freeze stored and delivered in adequate conditions to the different laboratories.

Assessment of the performance and health status of chickens was carried out by the Department of Animal and Food Science of the UAB (Spain).

The determination of the 17 polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) and the analysis of the 12 'dioxin-like' polychlorinated biphenyls (DL-PCBs), were performed in The Mass Spectrometry Laboratory/Dioxin Laboratory of the Ecotechnologies Department of the *Instituto de Investigaciones Químicas y Ambientales de Barcelona* (IIQAB) of the *Consejo Superior de Investigaciones Científicas* (CSIC) in Barcelona (Spain). Briefly, analysis were accomplished following well accepted procedures such as US EPA methods 1613 and 1668, which are based on the use of high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS) using ¹³C labelled PCDD/PCDFs and DL-PCBs as internal standards. Preliminary steps such as extraction and appropriate clean-up before final instrumental analysis were carried out.

Characterisation of polycyclic aromatic hydrocarbons (PAHs) and Polybromodiphenylethers (PBDEs) in feed and meat were done in the Environmental and Toxicological Laboratory, ISM-LPTC, UMR 5255 CNRS, Université de Bordeaux 1, Talence, France. These procedures involve a microwave-assisted extraction (MAE) of freeze-dried tissues, followed by purification on both alumina and silica (acidified silica for PBDEs) microcolumns and then by high performance liquid chromatography (HPLC) on an amino (NH₂) column. All analyses are performed by gas chromatography/mass spectrometry (GC/MS) for PAHs and by chromatography/electron capture detection (GC/ECD) for PBDEs, and quantification is carried out by a double level internal standard

method, with deuterated analogs for PAHs (Adapted from Baumard et al., 1997), and with PCB congeners for PBDEs (Adapted from Akutsu et al., 2001 and from Huwe et al., 2002).

The fatty acid (FA) composition (Guardiola et al., 1994), tocopherol/tocotrienol contents (Hewavitharana et al., 2004), polymer content (IUPAC 2508, 1992), p-anisidine value (AOAC official method) and TBARs value (Grau et al., 2000) were done by the Department of Nutrition & Food Science, University of Barcelona, Spain.

Table 2. Level of contaminants and degradation compounds of added fats in the experimental trial feeds.

Experiment	Treat	Added Fat 6%	Contaminant or degradation level of the added fat
1. Trans Fatty Acids (T)	HT	Palm FA distillate after hydrogenation	12.40 % ^A Total <i>Trans</i> Fatty Acids
	LT	Palm FA distillate	0.65 % ^A Total <i>Trans</i> Fatty Acids
2. Dioxins y PCBs (D)	HD	Fish oil	28.8 pg WHO-TEQ PCDD/Fs + DL-PCBs/g oil ^B
	LD	Fish oil	9.64 pg WHO-TEQ PCDD/Fs + DL-PCBs/g oil ^B
3. PAHs (P)	HP	Pomace-olive acid oil	5290 ng PAHs/g oil + PBDEs < 2 ng/g oil ^C
	LP	Olive acid oil	<18 ng PAHs/g oil + PBDEs < 2 ng/g oil ^C
4. Oxidación Products (O)	HO	Sunflower/olive oils (70:30 v/v) after frying	6.61 % Polymers 67.43 p-anisidina
	LO	Sunflower/olive oils (70:30 v/v)	0.35 % Polymers 2.74 p-anisidina

1H=high level; L=low level of contaminants and degradation compounds ^A AG= *Trans* Fatty Acids; ^B WHO-TEQ= toxic equivalent concentration; PCDD/Fs= polychlorinated dibenzo-dioxins/furans; DL-PCBs=Dioxin like-polychlorinated biphenyls; ^C PAH: polycyclic aromatic hydrocarbons (20 compounds); PBDs: Polybromodiphenylethers (4 compounds)

Department of Food Science (DI.S.A.), University of Bologna, Italy performed the analysis of *Trans* fatty acids (TFA; Buchgraber and Ulberth, 2001) and conjugated linoleic fatty acid isomers (CLA; Kramer et al., 1998).

The feed and meat analyses of Sterol composition (Larkeson et al., 2000) and Sterol oxidation products (SOPs; Hara and Radin, 1978, Ubhayasekera et al., 2004, Dutta & Appelqvist, 1997 and Dutta et al., 2002) were performed by the Department of Food Science, Swedish University of Agricultural Sciences, Uppsala, Sweden

Results and Discussion

The results on the inclusion of experimental fatty materials in poultry feeds at 6% show that neither chicken growth performances nor the results derived from the several health markers on animal health are affected. It may be possible that some of the differences found between treatments are more dependent on the type and profile of the fat, than on the alteration itself (Choque et al., 2007).

The **FA composition** in chicken meat changes according to the corresponding composition of the oil and feed included in the different trials (data not shown). Regarding the **α -tocopherol** (α -T), in general, their content in chicken meat is clearly influenced by the content of α -T in feeds. So, meats coming from the PAH (P) and oxidation (O) trial showed the higher values, between 8-12 mg α -T/kg, since fats added in these trials had vegetable origin. Meats coming from dioxin (D) and trans (T) trial showed maximum values around 4 mg/kg.

Only in the *Trans* trial (T), some remarkable differences in **Trans FA** content were found in feeds. So the treatment considered as High showed around 7% Trans FA in the lipid fraction, while there was only 0,5% in the Low treatment. As a consequence, only the poultry meats coming from the High treatment (HT) showed a certain % of trans FA in meat (3,5%), significantly different from the rest of the treatments (taking into account the four trials).

Regarding the **CLA content** in poultry meat, we only found significant values for the treatments of the Dioxin trial (D), since fish oils used in this trial were the only experimental fats showing values between 52 and 79 mg/100 g fat. As a consequence, meat showed values of 33 and 47 mg CLA/100 g lipids, corresponding to the LD and HD treatment, respectively.

The congener distribution profile (contribution in % of each congener to the total concentration in pg/g, not in pg WHO-TEQ/g) of chicken meat from the different experiments is very similar to the one observed in the corresponding feeds, both for **PCDD/Fs and DL-PCBs**. In addition, levels of the different PCDD/F and DL-PCB compounds increased when increasing the levels in the feed. Including skin together with the meat for the analysis raised the percentage of fat of the samples up to ~13% which was one of the factors that allowed us to obtain good quality analytical results, i.e. levels for most of the congeners determined were clearly above the detection limit of the method and, therefore, acceptable relative standard deviations (RSD%) were found between the different replicates. If the chicken meat samples from this study were commercialized and consumed with the skin they would be above the maximum levels established at the European Regulation for the summatory of PCDD/Fs+DL-PCBs, in the case of animals from Treatment LD, and both for PCDD/Fs and PCDD/Fs+DL-PCBs, in the case of Treatment HD. It has to be pointed out that for the corresponding chicken feeds, only the one for Treatment HD showed a level of PCDD/Fs+DL-PCBs above the maximum established at the European Directive for these kind of matrices (table 3).

Table 3. Levels of PCDD/Fs and DL-PCBs, expressed in pg WHO-TEQ (upperbound values), in feeds (/g) and chicken meat(/g fat) samples of the Dioxin experiment (D).

pg WHO-TEQ	Feed (/g) ¹		Chicken meat (/g fat) ²	
	LD	HD	LD	HD
PCDD/Fs	0.11	0.54	1.11 (6)	4.60 (8)
DL-PCBs	0.48	1.21	4.92 (7)	12.11 (8)
Sum (PCDD/Fs + DL-PCBs)	0.59	1.75	6.03 (7)	16.71 (8)

¹H=high level; L=low level of PCDD/Fs= polychlorinated dibenzo-dioxins/furans and DL-PCBs=Dioxin like-polychlorinated biphenyls

²Mean values of n=6 replicates are shown. In parenthesis relative standard deviations (RSD%) are included.

The **PAH** contents in the chicken thigh were extremely low or undetected (table 4). The rate of PAH transfer from feed to meat was very low, regarding the high capability of the animals to quickly metabolize PAHs.

Table 4. Levels of PAHs in ng/g (adapted from Baumard et al., 1997), in feeds and chicken meat samples of the PAHs experiment (P).

	Feed (/g) ¹		Chicken meat (/g fat) ²	
	LD	HD	LD	HD
Total PAHs³ ng/g	< 10	270	< 10	< 10

¹H=high level; L=low level of PAHs: polycyclic aromatic hydrocarbons.

²Mean values of n=5 replicates are shown.

³Total PAHs: sum of N, Acy, Ace, Fe, Phe, A, Fluo, Pyr, BaA, Triph+Chrys, BbF+BkF, BeP, BaP, Per, IP, DaA+DaC BP.

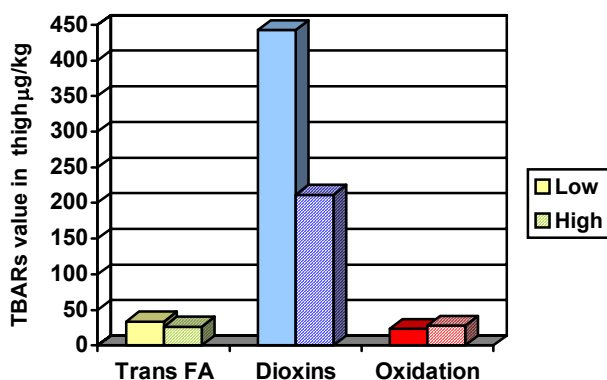
Although no particular supplementation of antioxidants was added to the feeds, in general all meat samples showed very low **TBA values**, included high oxidation level treatment (HO) (figure 1). However, significantly higher values were observed in the treatments corresponding to the Dioxin

trial (D), in which two different fish oils were used. Thus, we can conclude that the oxidability of the added fat is more important than the actual level of oxidation. Of the two treatments (LD and HD), meats showed TBA values significantly higher for the Low Dioxin treatment. This fact agrees with the higher values of oxidation that were previously detected in the fish oil used for the low dioxin treatment (HD:65 vs. LD:2819 μg MDA/kg feed).

Fats used in the four animal trials showed their characteristic pattern in cholesterol or other sterol contents. No differences were observed in **sterol and sterol oxides content** in chicken meat samples between treatments. These contents can be considered as very low values in general (from 43.2 to 74.4 mg cholesterol and from 0.01 to 0.86 mg cholesterol oxidation products per 100 g of thigh with skin).

In conclusion, the composition and level of alteration and/or contamination of feeding fats included at practical levels (6 %) have important consequences in chicken meat quality and must be taken into account. Results from the present project demonstrate the transference of harmful substances from fat feed to meat, specially in the case of **Trans fatty acids** and **PCDD/Fs and DL-PCBs**; the risk of exceeding the maximum levels established at the European Regulation for these contaminants in chicken meat should be kept in mind.

Figure 1. TBARs values (μg MDA/Kg thigh) of chicken meat samples of the Trans (T), Dioxins (D) and Oxidation (O) experiments.



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